Claims

1. Method for determining the presence of genetic element(s), such as nucleotide repeat(s), or marker(s) for microbial typing, in a nucleic acid sample, which method comprises the steps of:

a) providing the nucleic acid sample comprising the genetic element(s);

b) providing oligonucleotide(s) that are completely or partially complementary to the region(s) comprising the genetic element(s) of said nucleic acid sample;

c) annealing said oligonucleotide(s) to said nucleic acid sample;

d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme; and

e) detecting a ligation-by-product to determine whether a ligation reaction has occurred, as a measure of the presence of the genetic element(s),

wherein steps a)-e) are performed simultaneously or subsequently or in any combination of subsequent steps.

2. Method for analysing the number of nucleotide repeats in a nucleic acid sample, which method comprises the steps of:

a) providing a nucleic acid sample potentially comprising a nucleotide repeat;

b) providing oligonucleotide(s) complementary to said nucleotide repeat;

c) annealing said oligonucleotide(s) to said nucleic acid sample;

d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme; and

e) detecting a ligation by-product to determine whether a ligation reaction has occured, wherein steps a)-e) are performed simultaneously or subsequently or in any combination of subsequent steps.

3. Method for analysing the number of nucleotide repeats in a nucleic acid sample, which method comprises the steps of:

a) providing a nucleic acid sample potentially comprising a nucleotide repeat;

b) providing oligonucleotide(s) complementary to said nucleotide repeat;

c) annealing said oligonucleotide(s) to said nucleic acid sample;

d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme;

e) converting a ligation by-product into ATP; and

f) detecting said ATP to determine whether a ligation reaction has occured,

wherein steps a)-f) are performed simultaneously or subsequently or in any combination of subsequent steps.

4. Method for analysing the number of nucleotide repeats in a nucleic acid sample, which method comprises the steps of:

a) providing a nucleic acid sample potentially comprising a nucleotide repeat;

b) providing oligonucleotide(s) complementary to said nucleotide repeat;

c) annealing said oligonucleotide(s) to said nucleic acid sample;

d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme;

e) converting a ligation by-product into ATP; and

f) detecting said ATP by a luciferase-based assay as a measure of whether a ligation reaction has occured,

wherein steps a)-f) are performed simultaneously or subsequently or in any combination of subsequent steps.

5. Method for microbial typing of a nucleic acid sample, which method comprises the steps of:

a) providing a nucleic acid sample comprising at least one marker for microbial typing;

b) providing oligonucleotide(s) that are completely or partially complementary to the region(s) comprising marker(s) for microbial typing of said nucleic acid sample;

c) annealing said oligonucleotide(s) to said nucleic acid sample;

d) ligating said oligonucleotide(s) annealed to said nucleic acid sample to each other using a ligase enzyme; and

e) detecting a ligation by-product to determine whether a ligation reaction has occurred;

f) comparing the ligation pattern of the sample with a reference pattern, in order to determine the microbial type,

wherein steps a)-e) are performed simultaneously or subsequently or in any combination of subsequent steps.

6. Method according to any one of claims 1-5 wherein one of the oligonucleotides in step b) is adapted to anneal immediately outside the repeated sequence.
7. Method according to any one of claims 1-6 wherein the ligation by-product is AMP.
8. Method according to any one of claims 1-7 wherein step d) is performed employing a NAD^+-dependent DNA-ligase.
9. Method according to any one of claims 1-8 wherein step e) is performed employing a pyruvate phosphate dikinase.
10. Method according to any one of claims 1-6, wherein step d) is performed employing an ATP-dependent ligase, and apyrase is added to the ligation mixture of step d) before, during or after ligation in order to reduce excess amounts of DNA ligase substrate.
11. Method according to claim 10, wherein the ATP dependent ligase is T4 DNA ligase.
12. Method according to claim 10 or 11, wherein dATP is used as a substrate for the ATP dependent ligase in step d).
13. Method according to any one of claims 1-6 or 10-12, wherein the ligation by-product is PPi.
14. Method according to any one of claims 1-6 or 10-13, wherein step e) is performed employing a ATP-sulfurylase.
15. Method according to any one of claims 1-14, wherein the oligonucleotide employed is a mono-, di- or multimer of the repeat in itself.
16. Method according to any one of claims 1-14, wherein the oligonucleotides are complementary to, but that are out of phase with, said nucleotide repeat.
17. Method according to claim 16, further comprising a step wherein unannealed oligonucleotides are removed after the detection by using an exonuclease.
18. Method according to claim 16, further comprising a step wherein unannealed oligonucleotides are inactivated after the detection by using a phosphatase.

19 Method according to any one of claims 1-18, wherein the nucleic acid sample is immobilised on a support.

20. Method according to claim 19, further comprising a step wherein unannealed oligonucleotides are removed after the detection by washing.

21. Method according to any one of claims 1-20, preceeded by a step wherein the nucleic acid sample is amplified.

22. Method according to any one of claims 1-21, wherein the luciferase-based assay is a luminometric assay.

23. Method according to any one of claims 1-22, wherein the light that is produced in the luciferase reaction is enzymatically turned off after an initial level of produced light has been reached.

24. Method according to claim 23, wherein light production is turned off by the addition of apyrase.

25. Method according to any one of claims 1-24 where oligonucleotides complementary to a region outside that to be analyzed are used to generate a signal by ligation or primer extension that can be used to normalize the signal obtained from the region to be analyzed.

26. Kit for performing the method according to any one of claims 1-25 comprising, in separate vials, a ligase enzyme and an enzyme for converting a ligation by-product into ATP.

27. Kit according to claim 26 further comprising, in a separate vial, a luciferase enzyme.

28. Kit according to claim 26 or 27, further comprising, in a separate vial, apyrase.

29. Kit according to any one of claims 26-28, further comprising oligonucleotides complementary to a nucleotide repeat, optionally with an AdoPP5' modification, associated with a disease selected from the following group of diseases: Dentatorubral pallidoluysian atrophy (DRPLA), Fragile X syndrome, Fragile site FRAXE, Huntington´s disease, Kennedy´s disease, Machado-Joseph disease, Myotonic dystrophy, Friedrich's ataxia, Spinocerebellar ataxia type 1, Spinocerebellar ataxia type 2, Spinocerebellar ataxia type 3, Spinocerebellar ataxia type 6, Spinocerebellar ataxia type 8 and Spinocerebellar ataxia type 12.

30. Kit according to any one of claims 26-28, further comprising oligonucleotides complementary to a genetic region, optionally with an AdoPP5' modification, that is informative for identification of microbial species, from the following group: the *16S* rRNA gene, *23S* rRNA gene, *groEL, gyrB, rpoB, rnpB* and *groEL*, microsatellite and minisatellite sequences, VNTRs, the nuclear ribosomal DNA (rDNA) array – small-

subunit (SSU) (18S-like), large-subunit (LSU)(23S, 26S, or 28S-like), 5.8S rRNA genes, and internal transcribed ribosomal DNA (rDNA) spacers (ITS1 and ITS2).

31. Composition comprising a ligase enzyme and an enzyme for converting a ligation by-product into ATP.

32. Composition according to claim 31 further comprising a luciferase enzyme.

33. Composition according to claim 31 or 32 further comprising oligonucleotides complementary to a nucleotide repeat, optionally with an AdoPP5' modification, associated with a disease selected from the following group of diseases: Dentatorubral pallidoluysian atrophy (DRPLA), Fragile X syndrome, Fragile site FRAXE, Huntington´s disease, Kennedy´s disease, Machado-Joseph disease, Myotonic dystrophy, Friedrich's ataxia, Spinocerebellar ataxia type 1, Spinocerebellar ataxia type 2, Spinocerebellar ataxia type 3, Spinocerebellar ataxia type 6, Spinocerebellar ataxia type 8 and Spinocerebellar ataxia type 12.

34. Composition according to claim 31 or 32 further comprising oligonucleotides complementary to a genetic region, optionally with an AdoPP5' modification, that is informative for identification of microbial species, from the following group: the *16S* rRNA gene, *23S* rRNA gene, *groEL, gyrB, rpoB*, *rnpB* and *groEL*, microsatellite and minisatellite sequences, VNTRs, the nuclear ribosomal DNA (rDNA) array – small-subunit (SSU) (18S-like), large-subunit (LSU)(23S, 26S, or 28S-like), 5.8S rRNA genes, and internal transcribed ribosomal DNA (rDNA) spacers (ITS1 and ITS2).